A Strong Influence of Serotonin Axons on β-Adrenergic Receptors in Rat Brain

Abstract. *The role of serotonin axons in modulating the norepinephrine neurotransmission system in rat brain was investigated. Selective lesions of the forebrain serotonergic system were made by injecting 5,7-dihydroxytryptamine into the midbrain raphe nuclei. Four to six weeks after the lesion, the uptake of ^{3}H-labeled serotonin in the frontal cortex and the hippocampus was reduced by more than 90 percent, while neither the uptake of ^{3}H-labeled norepinephrine nor the content of norepinephrine was affected in either tissue. The number of β-adrenergic receptors, as measured by radioligand binding with ^{3}H-labeled dihydroalprenolol, was increased in the frontal cortex and hippocampus of rats with lesions. Similarly, specific lesions of central serotonin axons produced by systemically administered p-chloramphetamine resulted in an increase in the binding of ^{3}H-labeled dihydroalprenolol to β-adrenergic receptors and in the production of adenosine 3',5'-monophosphate in response to isoproterenol. These results indicate that serotonin axons may regulate β-adrenergic receptor number and function in brain.*

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Norepinephrine and serotonin neurotransmission systems in the brain have been implicated in central nervous system functions such as mood, sleep, arousal, learning, and neuroendocrine and autonomic nervous system regulation. These two neurotransmission systems usually have been studied independently, but recently the possible influences of one on the other have been considered (*1*).

The anatomical pathways for possible reciprocal interactions between the serotonin cell bodies in the midbrain raphe and the norepinephrine cell bodies in the locus coeruleus of the pons have been delineated (*2*). Drugs that act on norepinephrine receptors alter the firing rate of serotonin neurons (*3*) and the metabolism of serotonin (*4*), and lesions of the locus coeruleus increase serotonin fluorescence in the raphe (*5*). Conversely, lesions of the midbrain raphe with 5,6-dihydroxytryptamine or inhibition of serotonin synthesis with *p*-chlorophenylalanine result in a short-term (4-day) increase in tyrosine hydroxylase activity in the locus coeruleus (*6*).

Thus, it is reasonable to consider that a reciprocal functional link exists between the serotonin and norepinephrine systems. We report here that selective lesions of the serotonin axons innervating the forebrain of the rat result in large increases in the density of β-adrenergic receptors in the cerebral cortex and hippocampus and a concomitant increase in isoproterenol-stimulated production of adenosine 3',5'-monophosphate (cyclic AMP).

Male Sprague-Dawley rats (275 to 300 g) were anesthetized with pentobarbital (42 mg/kg) and injected with desmethylimipramine (25 mg/kg) 45 minutes before being positioned in a Kopf stereotaxic instrument. The serotonin-specific neurotoxin 5,7-dihydroxytryptamine–creatinine sulfate (5,7-DHT; Regis), in a dose equivalent to 7 μg of free base dissolved in 3 μl of vehicle (0.9 percent NaCl containing 0.01 percent ascorbic acid), was injected into both the dorsal and the median raphe nuclei over a 5-minute period (*7*). Control rats were treated identically except that no neurotoxin was injected. Other rats received an intraperitoneal injection of *p*-chloramphetamine–HCl (PCA, 5 mg/kg; Regis) on days 1, 3, and 5. Control rats were injected with saline at identical intervals. The animals were allowed to recover for 4 to 6 weeks (5,7-DHT) or 5 weeks (PCA) before being killed by decapitation.

At the time of decapitation, the high-affinity uptake of [^{3}H]serotonin and [^{3}H]norepinephrine was measured in homogenates from the frontal cortex and from the hippocampus as described (*8*). The content of norepinephrine in the frontal cortex and in the hippocampus was measured by high-performance liquid chromatography with electrochemical detection. The β-adrenergic receptor–mediated stimulation of cyclic AMP production by isoproterenol was carried out in minces of fresh hippocampus as described (*9*). The cyclic AMP produced was measured by radioimmunoassay with a Gammaflow automated system (*10*). The remaining frontal cortex and hippocampus were frozen on dry ice and stored at −80°C until assayed (*11, 12*) for β-adrenergic receptors with [^{3}H]dihydroalprenolol ([^{3}H]DHA), for α_1-adrenergic receptors with [^{3}H]prazosin, and for α_2-adrenergic receptors with *p*-[^{3}H]-aminoclonidine. Protein was measured as described (*13*) with bovine serum albumin as standard. Statistical comparisons were made by the grouped *t* test.

Lesions of the ascending serotonin systems by injections of 5,7-DHT into the raphe nuclei resulted in a decrease in high-affinity uptake of [^{3}H]serotonin of

Table 1. Effect of 5,7-dihydroxytryptamine (5,7-DHT) lesions on the uptake of [^{3}H]serotonin and [^{3}H]norepinephrine, content of norepinephrine, and binding to β- and α-adrenergic receptors in rat frontal cortex and hippocampus. Assays were performed 4 to 6 weeks after the injection of 5,7-DHT into both the dorsal and median raphe nuclei (*7*). The rats were injected intraperitoneally with desmethylimipramine (25 mg/kg) 45 minutes before injection of 5,7-DHT. For uptake experiments, the concentrations of [^{3}H]serotonin and [^{3}H]norepinephrine used were 29 n*M* and 55 n*M*, respectively. Binding to β-, α_1-, and α_2-adrenergic receptors was measured as described (*12*). Values are the mean ± standard error of the mean for the number of animals shown in parentheses.

Parameter	Brain region	Treatment	
		Control	5,7-DHT
[^{3}H]Serotonin uptake†	Frontal cortex	2727 ± 129 (24)	186 ± 19 (22)*
	Hippocampus	3015 ± 218 (17)	225 ± 24 (16)*
[^{3}H]Norepinephrine uptake†	Frontal cortex	606 ± 50 (13)	577 ± 53 (13)
	Hippocampus	989 ± 96 (15)	866 ± 60 (12)
Norepinephrine content‡	Frontal cortex	3.17 ± 0.29 (16)	2.73 ± 0.20 (17)
	Hippocampus	3.58 ± 0.25 (16)	3.99 ± 0.32 (16)
[^{3}H]Dihydroalprenolol binding§	Frontal cortex	93.2 ± 3.5 (20)	120.9 ± 3.2 (19)*
	Hippocampus	67.4 ± 3.6 (18)	92.1 ± 4.7 (19)*
[^{3}H]Prazosin binding§	Frontal cortex	171.9 ± 6.5 (6)	168.3 ± 3.0 (6)
	Hippocampus	62.3 ± 4.3 (8)	60.9 ± 3.2 (8)
p-[^{3}H]Aminoclonidine binding§	Frontal cortex	97.4 ± 7.9 (7)	88.8 ± 6.4 (9)

*$P < 0.001$ compared to control. †Values are femtomoles per milligram of protein per 4 minutes. ‡Values are nanograms per milligram of protein. §Values are femtomoles per milligram of protein.

more than 90 percent in both the frontal cortex and hippocampus (Table 1). In contrast, neither the uptake of [^{3}H]norepinephrine nor the content of norepinephrine in either the frontal cortex or the hippocampus was significantly affected by the 5,7-DHT injections (Table 1). Thus, the innervation of these forebrain structures by serotonin axons was nearly abolished by the lesions, whereas the norepinephrine axons were essentially intact. (As measured by these parameters, the serotonin lesions produced by the injections of 5,7-DHT into the midbrain raphe were much greater and more consistent than we have seen after intracerebroventricular administration of 5,7-DHT.)

The binding of [^{3}H]DHA to β-adrenergic receptors in the frontal cortex and hippocampus of rats lesioned with 5,7-DHT was significantly increased (Table 1). Scatchard analyses indicated that the number of β-adrenergic receptors (B_{max}) in these tissues was markedly increased after lesions with 5,7-DHT, whereas the receptor affinity (K_d) was not altered (Table 2).

Consistent with previous reports (*14*), injections of PCA reduced the uptake of [^{3}H]serotonin in the hippocampus by 65 ± 4 percent without reducing the uptake of [^{3}H]norepinephrine, indicating that this PCA treatment selectively lesioned serotonin axons. These lesions also resulted in a marked increase in the binding of [^{3}H]DHA to β-adrenergic receptors in the hippocampus (Fig. 1A). In addition, the isoproterenol-stimulated production of cyclic AMP, which is a functional response of the receptors, was similarly increased (Fig. 1B). The basal level of cyclic AMP was unaffected (Fig. 1B). In contrast to the increase in β-adrenergic receptors, binding of a saturating concentration of [^{3}H]prazosin to α_1-adrenergic receptors in the frontal cortex and the hippocampus and of *p*-[^{3}H]aminoclonidine to α_2-adrenergic receptors in the frontal cortex was not altered by lesions of serotonin neurons with 5,7-DHT (Table 1).

Since norepinephrine axons were intact after the lesions of the serotonin system with either 5,7-DHT or PCA, the increase in β-adrenergic receptors appears to be a response to the absence of serotonin axons. Thus this study indicates that serotonin axons exert a strong influence on, and may regulate, important components of norepinephrine neurotransmission—the β-adrenergic receptor and the attendant stimulation of cyclic AMP production—in the brain. Electrophysiological studies (*15*) have demonstrated that, 2 to 3 weeks after serotonin axons are lesioned with 5,7-DHT, neurons of the amygdala show increased responsiveness to norepinephrine. This too is indicative of an influence of serotonin axons on norepinephrine neurotransmission, possibly by a similar mechanism.

Table 2. Effect of 5,7-dihydroxytryptamine (5,7-DHT) lesions of the raphe nuclei on the density (B_{max}, femtomoles per milligram of protein) and affinity (K_d, n*M*) of [^{3}H]dihydroalprenolol ([^{3}H]DHA) binding to β-adrenergic receptors in rat frontal cortex and hippocampus. The rats were killed 4 weeks after lesions. Values (mean ± standard error of the mean) were determined by least-squares linear regression analyses of Scatchard plots (n = 8 to 9 for frontal cortex; n = 5 for hippocampus). The hippocampi from three rats were pooled for each analysis.

Treatment	[^{3}H]DHA binding	
	B_{max}	K_d
	Frontal cortex	
Control	154.6 ± 7.0	1.3 ± 0.1
5,7-DHT	201.6 ± 8.1*	1.6 ± 0.2
	Hippocampus	
Control	154.2 ± 9.9	6.0 ± 0.9
5,7-DHT	291.4 ± 25.0*	6.4 ± 0.3

*P < 0.001 compared to control.

The site of interaction between the serotonergic and noradrenergic systems that is responsible for the changes in β-adrenergic receptors after lesions of the serotonin axons has not been determined. Anatomical (*2*), biochemical (*6*), and physiological (*16*) studies indicate that serotonin axons from the midbrain raphe innervate the norepinephrine cell bodies in the locus coeruleus of the pons. However, this input is thought to be predominantly inhibitory (*6, 16*); thus it is not obvious how the absence of serotonin axons at the locus coeruleus could lead to an increase (up-regulation) in β-adrenergic receptors.

Alternatively, the mechanism by which serotonin neurons influence β-adrenergic receptors may involve local regulation in the forebrain at the level of the overlapping axon terminal projection fields of the raphe nuclei and locus coeruleus. For example, it is possible that serotonin axons influence norepinephrine neurotransmission by a specific action at the level of the β-adrenergic receptor. In fact, the lack of an effect of 5,7-DHT lesions on α_1- and α_2-adrenergic receptors favors this type of alternative and implies that the mechanism of the serotonin axon influence on norepinephrine neurotransmission is specific for the β-adrenergic receptor. This specificity may be achieved through an effect of a component of serotonin axons (either serotonin itself or a cotransmitter) on β-adrenergic receptor regulatory mechanisms at the level of either cell membrane events (increased insertion or decreased degradation of existing receptors) or earlier events (synthesis of new receptors).

Both serotonin and norepinephrine neurotransmission have been implicated in depression as well as in other central nervous system disorders (*17*). In particular, much work has focused on the involvement of β-adrenergic receptors in depression and in the mechanisms of action of antidepressant treatments (*18*). Both antidepressant drugs and electroconvulsive shock decrease (down-regulate) β-adrenergic receptors in rat brain (*19*), while reserpine, a drug capable of causing depression in humans (*20*), increases the number of β-adrenergic receptors in rat brain (*12, 21*). Furthermore, in brains from suicide victims, presynaptic markers for serotonin neurotransmission are reported to be decreased (*22*) and β-adrenergic receptors are reported to be increased (*23*). Thus, the regulation of the number of brain β-adrenergic receptors by serotonin axons is possibly a critical link between these two neurotransmission systems. Since norepinephrine axons are anatomically intact after these lesions of the serotonin system, this model may be useful in testing and understanding the effects of antidepressant treatments on the regulation of increased numbers of β-adrenergic receptors in brain.

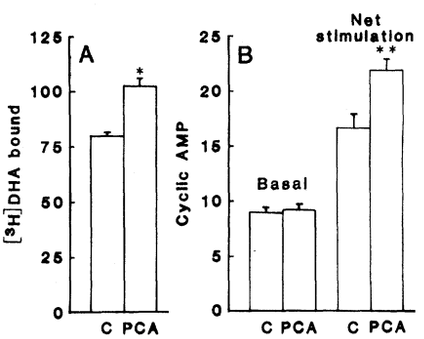

Fig. 1. Effect of lesions of serotonin axons with *p*-chloramphetamine (PCA) on (A) the binding of [^{3}H]dihydroalprenolol ([^{3}H]DHA, femtomoles per milligram of protein) to β-adrenergic receptors and (B) isoproterenol-stimulated production of cyclic AMP (picomoles per milligram of protein) in the hippocampus. The concentration of [^{3}H]DHA used was 5.9 n*M*. β-Adrenergic receptor–mediated stimulation of cyclic AMP was determined by incubating hippocampal minces with 10 μ*M* L-isoproterenol for 10 minutes in the presence of 50 μ*M* isobutylmethylxanthine. Net stimulation equals total cyclic AMP in the tissue after incubation with isoproterenol minus the basal level. Values represent the mean ± standard error of the mean (n = 15 to 16). (*) P < 0.001, (**) P < 0.01 compared to control (C).

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Induction of Autoimmune Thyroiditis in Chickens by Dietary Iodine

Abstract. *Clinical studies have suggested that excess dietary iodine promotes autoimmune thyroiditis; however, the lack of a suitable animal model has hampered investigation of the phenomenon. In this study, different amounts of potassium iodide were added to the diets of chicken strains known to be genetically susceptible to autoimmune thyroiditis. Administration of iodine during the first 10 weeks of life increased the incidence of the disease, as determined by histology and the measurement of autoantibodies to triiodothyronine, thyroxine, and thyroglobulin. Further support for the relation between iodine and autoimmune thyroiditis was provided by an experiment in which iodine-deficient regimens decreased the incidence of thyroid autoantibodies in a highly susceptible strain. These results suggest that excessive consumption of iodine in the United States may be responsible for the increased incidence of autoimmune thyroiditis.*

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Clinical studies have suggested an association between increased consumption of iodine and a sharp rise in the incidence of autoimmune thyroiditis in recent years. For example, the prevalence of histologic thyroiditis in thyroidectomy specimens was found to be low in iodine-deficient areas in the United States (*1*) and to increase after the introduction of iodine prophylaxis (*2*). Other clinical studies revealed that treatment with iodine or iodine-containing agents increased the incidence of thyroid autoantibodies (*3*, *4*). Conversely, treatment of animals with iodine alone did not cause autoimmune thyroiditis (*5*). Interestingly, large parenteral doses of iodine accompanied by chronic stimulation with thyrotropin caused inflammatory changes in the thyroid gland of hamsters (*6*). The apparent contradiction between the clinical and animal data prompted the present study. In addition, it seemed important to develop an appropriate animal model for studying the relation between iodine and autoimmune thyroiditis. We report that dietary iodine induced autoimmune thyroiditis in genetically susceptible chickens and that iodine depletion had an ameliorative effect.

We chose two genetically susceptible chickens as our experimental models in view of the well-known involvement of genetic factors in autoimmune thyroid disease. The Cornell C strain (CS) has a low incidence of mild autoimmune thyroiditis through the first several months of life, but by 1 year 25 percent of females have autoantibodies to triiodo-

Table 1. Effect of dietary iodine on autoantibody responses in CS chickens. Newly hatched CS chicks were maintained on a normal diet and water with or without added KI. Thyroglobulin autoantibody titers were determined by passive hemagglutination (*20*). Antibodies to T_3 and T_4 were assayed by an electrophoretic-autoradiographic technique (*7*). Chicken serum (50 μl) was incubated with 200 nCi (1 μl) of [^{125}I]T_3 or [^{125}I]T_4 (specific activity, 750 to 1250 μCi/μg; New England Nuclear) at 37°C for 30 minutes. Electrophoresis was performed on 125 by 245 mm polyester film (Gel Bond, FMC Corp.) using 1 percent agarose (High M_r, Bio Rad) in 0.15M tris-HCl (*p*H 8.0) at 10°C and 15 V/cm for 2 hours. The slabs were dried under a stream of warm air and overlayed on Kodak XAR-5 film for 16 hours. The autoradiographs were aligned with the dried gels and the distribution of labeled hormone was determined by cutting out and counting the radioactivity in the appropriate regions. Reproducibility was ≤4 percent. Mean values for antibody-positive birds at 10 weeks of age were 18.9 percent T_3 antibody and 26 percent T_4 antibody. The data were subjected to chi-square analysis with Bonferroni correction (*21*); chicks treated with either dose of iodide did not differ from each other, but the treated groups together had significantly higher titers of antibodies to Tg, T_3, and T_4 than the untreated group commencing at 6 weeks of age ($P < 0.05$). Values are numbers of chicks.

Treatment	Incidence of Tg antibody*				Incidence of T_3 and T_4 antibodies at 10 weeks†
	4 weeks	6 weeks	8 weeks	10 weeks	
None	0 of 42	1 of 42	0 of 42	0 of 42	2 of 42
Iodine (2 mg/dl)	0 of 44	7 of 44	6 of 44	4 of 44	12 of 44
Iodine (20 mg/dl)	1 of 44	12 of 44	6 of 44	8 of 44	12 of 44

*Log_2 titer, >3 by passive hemagglutination. †At least 0.5 percent of the total radiolabeled T_3 or T_4 bound to γ-globulin.